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EFFECTS OF DIGESTATE FROM SWINE MANURE DIGESTER ON *IN VITRO* GROWTH OF CROP FUNGAL PATHOGENS: A LABORATORY STUDY

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ABSTRACT. *Anaerobic digestion is one of the most popular methods for swine manure treatment in China, and the resultant digestates are mainly used as fertilizer on arable land. Residues from anaerobic fermentation may be used to mitigate the use of chemical fungicides, but relevant information is lacking. In this lab-scale study, original digestate (OD) from a swine manure-fed digester and centrifuged supernatant liquid (SL) with different storage times (0, 7, 14, or 28 d) were added to potato dextrose agar (PDA) media at a rate of 5% to investigate the effects on* in vitro *mycelial growth of seven phytopathogenic fungi:* Fusarium oxysporum*,* Sclerotinia sclerotiorum*,* Rhizotonia cerealis*,* Bipolaris sorokinianum*,* Rhizoctonia solani*,* Exserohilum turcicum*, and* Bipolaris maydis*. Diameters of the fungal colonies were measured at 1 d intervals for 7 consecutive days, and the absolute growth rate (AGR) and growth coefficient (k) were calculated. Results showed that the colony sizes of* Fusarium oxysporum*,* Sclerotinia sclerotiorum*,* Rhizotonia cerealis*,* Bipolaris sorokinianum*, and* Rhizoctonia solani *on the OD-treated media were significantly smaller ($p < 0.01$) than the corresponding controls regardless of the storage time. Similarly, independent of storage time, SL-treated media were shown to significantly ($p < 0.01$) suppress AGR compared to the controls for all seven fungi except for* Exserohilum turcicum*, where no significant difference was observed between the 14-day-old SL treatment and control. The average k values of the fungi on the OD-treated media ranged from 29% to 143% of the values on the SL-treated media. The results of this study suggest potential use of digestate for plant disease control, which would reduce the use of chemical fungicides. Further studies are needed to investigate the fungicidal mechanism and fungicidal efficacy of OD and SL under field conditions.*

Keywords. *Biofungicide, Crop, Digestate, Inhibitory effect, Phytopathogen, Swine manure.*

China is the largest hog producer in the world, and proper management of animal manure is imperative for sustainable development of Chinese hog production. Anaerobic digestion (AD) is one of the most widely used technologies for pig manure treatment throughout the world (DeSutter and Ham, 2005; Bernet and Béline, 2009; Bortone, 2009) because AD is considered an eco-friendly process in terms of organic matter degradation, odor reduction, and energy recovery. AD technology has found wide adoption in China. AD inevitably produces residue or digestate that contains high levels of soluble ammonium nitrogen (NH_4-N) and that can be applied directly onto cropland as a high-value fertilizer or soil conditioner (Weiland, 2010). The solid portion of the digestate can be transported, while the liquid portion can be applied *in situ* (Paavola and Rintala, 2008). In China, land application of liquid digestate is preferred due to lack of technology and equipment for liquid digestate disposal. However, there is not always sufficient land surrounding pig farms for liquid digestate assimilation. Hence, alternative methods for liquid digestate disposal or utilization are desired.

Numerous publications have shown the positive effects of compost application on the reduction of plant diseases (Eo and Park, 2013; Mehta et al., 2014). Water extracts from anaerobically incubated composts of spent mushroom substrate were shown to significantly reduce the scab area on the leaves of apple trees and the *in vitro* germination of conidia of the apple scab pathogen *Venturia inaequalis*, and increase the efficacy of extracts from manure-amended compost (Yohalem et al, 1996a, 1996b). The inhibitory principle might be attributed to the metabolite produced by anaerobic microorganisms in the compost (Cronin et al., 1996). Bustamante et al. (2012) observed strong *in vitro* suppression of *Fusarium oxysporum* f. sp. *melonis* induced by compost of the solid fraction of digestate from feedstocks of cattle slurry and vine shoot prunings. Plöchl et al. (2014) found that the dwell time for inactivation of 90% of phytopathogens in both lab-scale digesters and full-scale biogas plants ranged from 0.13 to 96 h, and phytopathogens showed shorter inactivation time in ensiled material than in

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fresh material. In a laboratory study, López-Robles et al. (2013) observed that liquid swine manure from a storage lagoon, after partial anaerobic incubation, significantly decreased cyst nematode populations and egg hatching percentages. Whether or not the anaerobic process is combined with composting, the products exhibit suppressive effects on phytopathogens. It can be inferred that digestate may have potential value-added properties as a biofungicide.

Biofungicides are a viable alternative to chemical fungicides. Biofungicides are natural microbial or biochemical products, and some biochemical biopesticides may be products of fermentation (McGrath, 2004). It is extrapolated that digestate from anaerobic fermentation, especially from digesters with animal manure feedstock, may partly replace chemical fungicides for fungal phytopathogen control. However, information is lacking about the inhibitory effects of digestate on plant fungal pathogens. Therefore, digestate from a biogas plant with pig manure feedstock was used in this laboratory-scale study to (1) investigate the *in vitro* growth of phytopathogenic fungi on digestate-added culture media and (2) evaluate the inhibitory effects of AD residues on fungal phytopathogens.

MATERIALS AND METHODS

ANAEROBICALLY DIGESTED PIG SLURRY

The digestate used in this study was obtained from a biogas plant using an upflow anaerobic solid reactor (USR) in the southern suburbs of Beijing, China. The USR has a capacity of 250 m^3 and was fed with swine manure. Fresh digestate was collected in plastic containers and stored at room conditions (temperature range of 21°C to 24°C) during the experimental period. On each trial day (i.e., on the day of arrival and at 7, 14, and 28 days of storage under the lab conditions), approximately 400 mL of digestate was taken from the containers. Half (200 mL) of the original digestate (OD) was applied to culture media, and the other half (200 mL) was first centrifuged at 10,000 rpm for 10 min (model 3K15, Sigma-Aldrich Co., Osterode, Germany) and then the resulting supernatant liquid (SL) was applied to culture media. The application rate and media preparation are described in the following sections.

INOCULUM PREPARATION

Seven phytopathogenic fungi, including *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizotonia cerealis*, *Bipolaris sorokinianum*, *Rhizoctonia solani*, *Exserohilum turcicum*, and *Bipolaris maydis*, were procured from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. These fungi can infect crops and cause soybean root rot, soybean sclerotinia stem rot, wheat sharp eye spot, wheat root rot, rice sheath blight, corn northern leaf blight, and corn southern leaf blight, respectively. Each fungus was inoculated into a potato-dextrose-agar (PDA) medium on Petri plates for rejuvenation. The rejuvenated fungal colonies were used as inoculum 4 to 5 days later.

MEDIUM PREPARATION

The PDA medium (Beijing Aoboxing Biotech Co., Ltd., Beijing, China) was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C for 20 min. When the temperature of the autoclaved medium dropped to 40°C to 50°C, OD or SL were added separately to the melted PDA medium at a rate of 5%, and sterile distilled water was applied at the same rate as the control. After thorough mixing, the media were poured onto Petri plates. For each of the seven pathogenic fungi, five replicates (agar plates), each containing 15 mL of mixed medium, were prepared for both treatments (OD and SL). The control group had three replicates.

INOCULATION AND CULTURE CONDITIONS

On each trial day, the rejuvenated fungal colonies were taken out of the incubator. Mycelial pads of 5 mm diameter were removed from the leading edge of actively growing colonies with a stainless steel borer and put upside-down on the center of the experimental plates with culture media, one agar pad per plate. The plates inoculated with *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizotonia cerealis*, and *Rhizoctonia solani* were incubated at a species-specific temperature of 25°C for 7 d, and the plates inoculated with *Bipolaris sorokinianum*, *Exserohilum turcicum*, and *Bipolaris maydis* were cultured at a species-specific temperature of 28°C for 7 d. If the mycelium grew fast and occupied the whole plate within 7 d, the Petri plates were removed from the incubator and the trial ended immediately. The entire experimental period lasted 35 d.

The inoculation and measurement of each fungus were performed in a biosafety level 1 microbiology analysis laboratory at our institute. The laboratory room consisted of three parts, including the entrance, a buffer booth, and an operational area. To ensure biosafety, operators must change shoes and put on lab coats and gloves in the buffer booth before entering the operational area. The reverse procedure is followed when leaving the laboratory. Exhaust air of the lab room was filtered before going to the atmosphere, and the room was disinfected by ultraviolet lamp after each use. All the fungi were inactivated by autoclaving upon completion of the experiment.

DATA COLLECTION AND ANALYSIS

The diameter (D) of the control and treatment colonies of each fungus was measured every 24 h using vernier calipers for 7 d. Absolute growth rate (AGR, cm d^{-1}) of mycelium was calculated using the following equation:

$$\mathrm{AGR} = \frac{D - 0.5}{t} \quad (1)$$

where

D = diameter of fungal colony (cm)

0.5 = diameter of inoculated pad (cm)

t = incubation time (d).

The mycelial diameter of each fungus as a function of time was plotted, and the logarithmic phase of each growth curve was fitted with an empirical equation. The equation was then used to calculate the time (t_d) of doubling the my-

celial area. Finally, the growth coefficient (k, d^{-1}) of each fungus on different culture media was estimated from the following equation:

$$k = \frac{\ln 2}{t_d} \quad (2)$$

All the data were subjected to analysis of variance (ANOVA), and the differences were examined by two-tailed t-tests. All analyses were performed using SPSS (release 10.0, SPSS, Inc., Chicago, Ill.). Means were considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

VARIATION OF DIGESTATE CHARACTERISTICS DURING STORAGE

The characteristics of the digestate at different storage times are shown in table 1. For both OD and SL, the contents of NH_4^+-N, total nitrogen (TN), COD, and total organic carbon (TOC) changed with storage time. The TN, COD, and TOC concentrations of OD were higher than those of SL, but a significant difference ($p < 0.05$) was only observed for COD. The decreased NH_4^+-N concentrations in OD after storage might be due to volatilization of NH_3 during storage. The NH_4^+-N values of stored OD were lower than those of the corresponding SL, which might be explained by the lesser volume of SL and soluble ammonia dissolving in the liquid fraction of the digestate. The decline in COD content of the digestate after storage might be attributed to microbial activity, and the difference in COD concentration between OD and SL might have resulted from sedimentation of indissolvable organic matter by centrifuging.

EFFECTS OF ORIGINAL DIGESTATE ON *IN VITRO* GROWTH OF FUNGI

The diameters of fungal colonies on digestate-laden PDA media are shown in table 2. The colony sizes of *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizotonia cerealis*, *Bipolaris sorokinianum*, and *Rhizoctonia solani* on the digestate-laden media were significantly smaller ($p < 0.01$) than those of the corresponding controls regardless of digestate storage time. The same held true with AGR for all fungi except for *Bipolaris maydis* (as shown in fig. 1), in which case no significant difference was observed between 28-day-old OD and the control.

Both *Sclerotinia sclerotiorum* and *Rhizoctonia solani* grew fast. The mycelium in the control groups spread over the whole plate surface within 3 d, and the measurement was ceased accordingly; hence, no profile is shown due to early suspension of the trial. The maximal AGR of the treatment groups was 0.76 and 1.00 cm d^{-1} for *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, respectively, whereas the peak values of the corresponding controls were 2.81 and 2.77 cm d^{-1}, respectively. For *Exserohilum turcicum*, *Rhizotonia cerealis*, *Bipolaris sorokinianum*, *Exserohilum turcicum*, and *Bipolaris maydis*, AGR peaked within 3 d of incubation, exception for the control group of *Rhizotonia cerealis* and the treatments with 0 and 14 d storage for *Bipolaris maydis*.

The suppressive effect of OD on the growth of pathogenic fungi may be partially attributed to ammonia, a fungitoxic compound (Suárez-Estrella et al., 2013). Lyimo et al. (2012) reported that maize fertilized with composted cattle manure had lower levels of gray leaf spot, and the higher NH_4^+-N leaf tissue concentration might have inhibited or interfered with the development of the pathogen in leaf tissues. The high ammonia concentrations of the digestate were beneficial to the suppression of fungal colonies. The C/N ratios (ranging from 1.9 to 3.0) of the digestate might be another factor affecting mycelial growth in that relatively low C/N ratios are adverse to the growth of phytopathogenic fungi. The C/N ratios of the digestate were close to that (4.56) of liquid swine manure applied for soil disinfestation of *Globodera rostochiensis* populations in laboratory conditions (López-Robles et al., 2013). However, the production of abiotic and biotic substances during anaerobic fermentation may also be fungicidal to pathogens, and the mechanism needs further elucidation.

EFFECTS OF SUPERNATANT LIQUID OF DIGESTATE ON *IN VITRO* GROWTH OF FUNGI

The diameters of fungal colonies on SL-treated PDA media are shown in table 3, and the AGR profiles of the studied fungi during the entire trial period are shown in figure 2. The colony sizes of *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizotonia cerealis*, *Bipolaris sorokinianum*, and *Rhizoctonia solani* on the SL-treated media were significantly smaller ($p < 0.01$) than those of the corresponding controls regardless of digestate storage time. Compared to the controls, the SL-treated media significantly ($p < 0.01$) suppressed the AGR of all fungi except for *Exserohilum turcicum*, in which case no significant differ-

Table 1. Characteristics of digestate and its centrifuged liquid at different storage time (mean ±SD).

Storage Time (d)	Digestate[a]	NH_4^+-N (mg L^{-1})	TN (mg L^{-1})	COD[b] (mg L^{-1})	TOC (mg L^{-1})	pH	TOC/TN
0	OD	5646 ±101	8262 ±220	64520 ±9780	17500 ±892	7.7 ±0.1	2.1
	SL	5151 ±357	7720 ±157	36710 ±13957	17340 ±321	7.8 ±0.1	2.2
7	OD	4573 ±392	6860 ±643	33330 ±5562	16960 ±439	7.6 ±0.1	2.5
	SL	5615 ±231	7440 ±268	24690 ±1202	14200 ±997	7.9 ±0.2	1.9
14	OD	4831 ±711	7570 ±196	40780 ±2748	17250 ±507	7.5 ±0.1	2.3
	SL	5541 ±135	7530 ±91	29140 ±2195	15120 ±335	7.8 ±0.1	2.0
28	OD	5049 ±275	5540 ±251	36340 ±10161	13880 ±1402	7.8 ±0.2	2.5
	SL	5524 ±161	5020 ±404	23340 ±3007	15140 ±607	7.8 ±0.1	3.0

[a] OD = original digestate from swine manure-fed digester was added to PDA media directly. Part of the OD was centrifuged, and the resultant supernatant liquid (SL) was applied to PDA culture media.

[b] There were significant differences ($p < 0.05$) in COD between OD and SL for all storage times.

Table 2. Colony sizes of crop fungi (mean ±SD) on original digestate-added media.[a]

Phytopathogen	Digestate Storage Time (d)	Diameter of Fungal Colonies (cm) at Different Post-Digestate Application Times						
		1 d	2 d	3 d	4 d	5 d	6 d	7 d
Fusarium oxysporum	0	0.63 ±0.02**	0.79 ±0.06**	0.88 ±0.05**	0.93 ±0.09**	0.96 ±0.13**	1.06 ±0.26**	1.27 ±0.55**
	7	0.91 ±0.08**	1.48 ±0.14**	1.94 ±0.15**	2.00 ±0.15**	2.26 ±0.19**	2.42 ±0.23**	2.58 ±0.35**
	14	1.08 ±0.03**	1.90 ±0.13**	2.54 ±0.32**	3.28 ±0.40**	3.42 ±0.58**	3.45 ±0.55**	3.36 ±0.56**
	28	0.68 ±0.03**	1.76 ±0.12**	2.36 ±0.14**	3.01 ±0.32**	3.15 ±0.55**	3.22 ±0.56**	3.35 ±0.64**
	Control	1.47 ±0.37	2.95 ±0.46	4.22 ±0.56	5.45 ±0.45	6.35 ±0.54	7.18 ±0.38	8.07 ±0.42
Sclerotinia sclerotiorum[b]	0	0.50 ±0.00**	0.76 ±0.21**	0.93 ±0.43**	-	-	-	-
	7	0.51 ±0.01**	2.01 ±0.16**	2.11 ±0.06**	-	-	-	-
	14	0.50 ±0.00**	0.79 ±0.20**	1.82 ±0.50**	-	-	-	-
	28	0.50 ±0.00	0.50 ±0.00**	0.50 ±0.00**	-	-	-	-
	Control	1.56 ±0.71	6.22 ±0.95	8.81 ±034	-	-	-	-
Rhizotonia cerealis	0	0.52 ±0.01**	0.55 ±0.05**	0.55 ±0.04**	0.54 ±0.03**	0.53 ±0.01**	0.53 ±0.01**	0.48 ±0.15**
	7	0.50 ±0.01	0.74 ±0.13*	0.79 ±0.12**	0.79 ±0.12**	0.79 ±0.12**	0.79 ±0.12**	0.79 ±0.12**
	14	0.54 ±0.09**	0.60 ±0.13**	0.67 ±0.19**	0.70 ±0.27**	0.76 ±0.39**	0.76 ±0.39**	0.76 ±0.39**
	28	0.50 ±0.00	0. 50 ±0.00**	0.50 ±0.00**	0.50 ±0.00**	0.50 ±0.00**	0.50 ±0.00**	0.57 ±0.15**
	Control	0.89 ±0.23	1.55 ±0.29	2.34 ±0.39	3.10 ±0.44	3.89 ±0.33	4.57 ±0.30	5.37 ±0.39
Bipolaris sorokinianum	0	0.55 ±0.01**	0.78 ±0.11**	0.76 ±0.08**	0.82 ±0.12**	0.84 ±0.12**	0.86 ±0.12**	0.89 ±0.16**
	7	0.54 ±0.04**	0.72 ±0.14**	0.77 ±0.17**	0.79 ±0.17**	0.83 ±0.19**	0.86 ±0.22**	0.92 ±0.26**
	14	0.50 ±0.01**	0.99 ±0.13**	1.35 ±0.08**	1.53 ±0.06**	1.68 ±0.13**	1.77 ±0.20**	1.70 ±0.15**
	28	0.50 ±0.00**	0.73 ±0.10**	1.09 ±0.25**	1.33 ±0.24**	1.38 ±0.27**	1.41 ±0.24**	1.43 ±0.25**
	Control	1.34 ±0.27	2.56 ±0.30	3.81 ±0.50	4.83 ±0.30	5.91 ±0.43	6.75 ±0.66	7.54 ±1.01
Rhizoctonia solani[b]	0	0.79 ±0.17**	1.01 ±0.30**	1.37 ±0.80**	-	-	-	-
	7	0.69 ±0.05**	1.15 ±0.42**	1.48 ±0.85**	-	-	-	-
	14	1.16 ±0.32**	2.55 ±0.33**	3.49 ±0.64**	-	-	-	-
	28	0.73 ±0.10**	1.55 ±0.40**	1.97 ±0.73**	-	-	-	-
	Control	2.21 ±0.44	5.95 ±0.72	8.82 ±0.29	-	-	-	-
Exserohilum turcicum	0	0.50 ±0.00**	0.61 ±0.14	0.74 ±0.33	0.77 ±0.33	0.79 ±0.33	0.81 ±0.35	0.89 ±0.42
	7	0.63 ±0.02*	0.73 ±0.08**	0.79 ±0.14**	0.81 ±0.17**	0.80 ±0.18**	0.81 ±0.18**	0.81 ±0.18**
	14	0.57 ±0.07	0.84 ±0.09*	1.21 ±0.19*	1.38 ±0.12*	1.49 ±0.10*	1.52 ±0.12*	1.52 ±0.12*
	28	0.56 ±0.08	0.84 ±0.17**	1.25 ±0.14**	1.29 ±0.09**	1.47 ±0.09**	1.57 ±0.04**	1.60 ±0.05**
	Control	0.77 ±0.22	1.38 ±0.67	2.06 ±1.25	2.42 ±1.47	2.60 ±1.67	2.64 ±1.65	2.69 ±1.62
Bipolaris maydis	0	0.51 ±0.02**	0.63 ±0.17	0.63 ±0.16*	0.67 ±0.14**	1.10 ±0.35	1.16 ±0.39	1.28 ±0.37
	7	0.55 ±0.04*	0.75 ±0.12**	0.96 ±0.08	1.09 ±0.07	1.12 ±0.08*	1.20 ±0.10*	1.25 ±0.11*
	14	0.51 ±0.01	0.78 ±0.13	1.04 ±0.15	1.26 ±0.16	1.34 ±0.16	1.51 ±0.24	1.66 ±0.27
	28	0.59 ±0.02	0.91 ±0.04	1.41 ±0.05	1.54 ±0.08	1.81 ±0.10	1.91 ±0.11	1.95 ±0.07
	Control	0.62 ±0.07	0.94 ±0.13	1.21 ±0.18	1.40 ±0.19	1.55 ±0.31	1.66 ±0.32	1.81 ±0.39

[a] Significance: * = significant difference between OD-treated media and the control at $p < 0.05$; ** = highly significant difference between OD-treated media and the control at $p < 0.01$.

[b] Trials were suspended soon after the fungal colonies of the control group spread over the whole Petri plate.

ence was observed between 14-day-old SL and the control. For *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, the control mycelia grew fast and covered the whole plated within 3 d. Therefore, no profile is shown due to early suspension of the trial.

The SL storage time had an impact on the mycelial growth of different fungi. For instance, the AGR of *Fusarium oxysporum*, *Rhizotonia cerealis*, *Bipolaris sorokinianum*, and *Exserohilum turcicum* was lowest on media supplemented with SL stored for 28 d, 28 d, 28 d, and 0 d, respectively.

The inhibitory effects of SL might have been associated with the SL ammonium content, since the stored SL had higher NH_4^+-N content than the corresponding OD. Other abiotic substances produced during anaerobic digestion, such as volatile fatty acids, may be fungicidal to phytopathogens as well. Volatile fatty acids are produced during anaerobic digestion as intermediates of the biochemical process (Weiland, 2010). Anaerobic incubation increases the volatile fatty acids in liquid swine manure, which were reported to be the dominant lethal agent for cyst nematode (*Globodera rostochiensis*) populations in acidic soils (López-Robles et al., 2013). However, in the current study, the volatile fatty acids in the digestate were not analyzed. This analysis will be included in future studies.

COMPARISON OF INHIBITORY EFFECTS OF ORIGINAL DIGESTATE AND SUPERNATANT LIQUID

The growth coefficients (k) of the seven fungi on different culture media are shown in table 4. Except for *Rhizoctonia solani* and *Exserohilum turcicum*, the k values of fungi on OD-treated media were greater than those of fungi on SL-treated media if digestate with 0-d storage was used. The k values of *Fusarium oxysporum* on OD-treated media were greater than those of fungi on SL-treated media regardless of storage time. The averaged k values of fungi on OD-treated media were 29%, 88%, 50%, 43%, 143%, 94%, and 68% of the values on SL-treated media for *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizotonia cerealis*, *Bipolaris sorokinianum*, *Rhizoctonia solani*, *Exserohilum turcicum*, and *Bipolaris maydis*, respectively.

The differences in AGR between OD-treated media and SL-treated media with different storage times are also shown in table 4. The inhibitory effects on *Fusarium oxysporum*, *Bipolaris sorokinianum*, and *Bipolaris maydis* were significantly higher ($p < 0.05$) for OD than for SL, except that *Bi-*

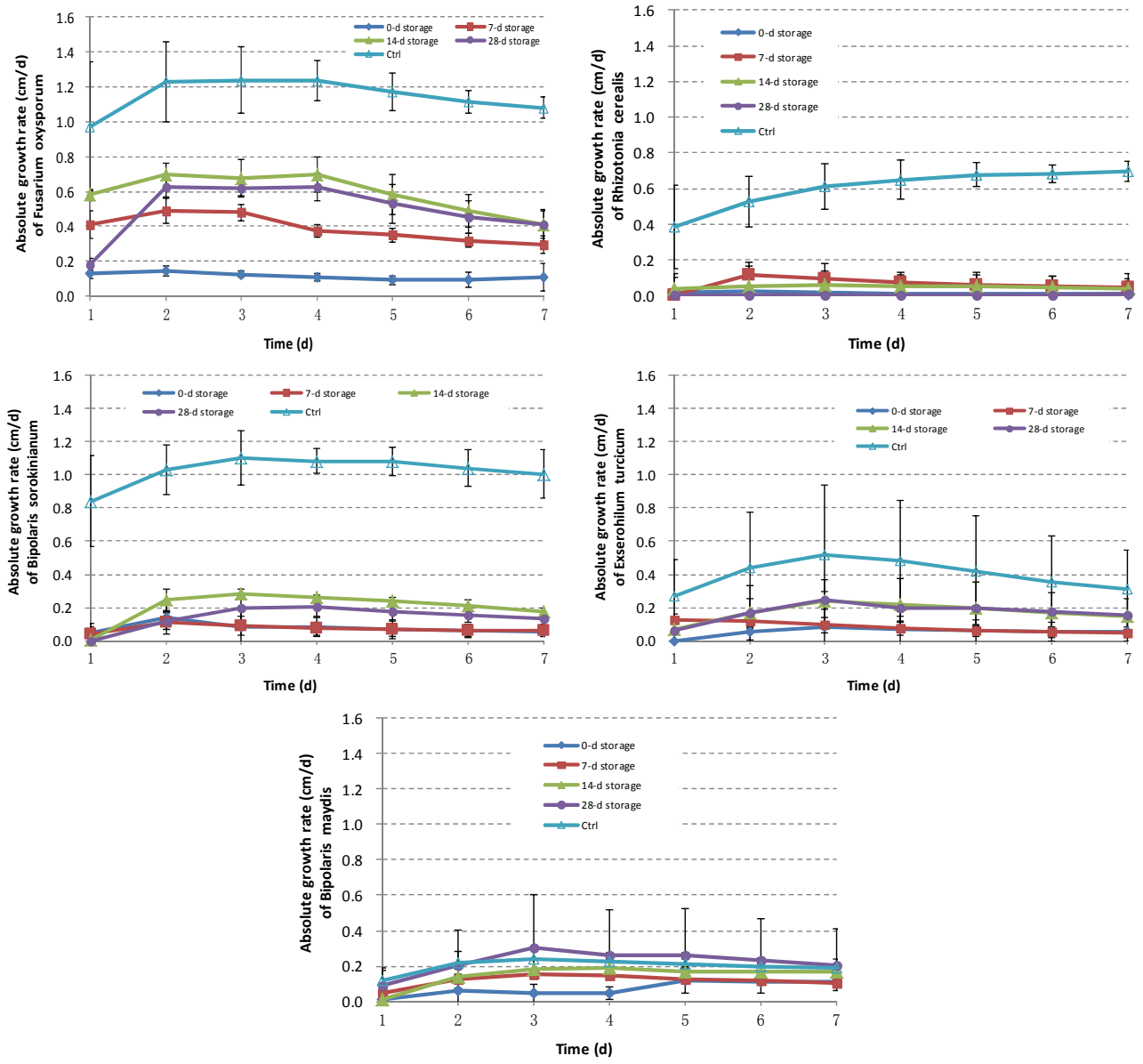

Figure 1. Profiles of absolute growth rates (AGR) of *Fusarium oxysporum*, *Rhizotonia cerealis*, *Bipolaris sorokinianum*, *Exserohilum turcicum*, and *Bipolaris maydis* on the original digestate-treated media.

polaris maydis had the reverse result when OD and SL were stored for 28 d. For *Rhizotonia cerealis*, significant differences ($p < 0.05$) in AGR between OD and SL were observed with storage times of 0 d and 14 d. For *Exserohilum turcicum*, significant differences ($p < 0.01$) in AGR between OD and SL were observed only with a storage time of 14 d. There were no significant differences in AGR between OD and SL for both *Sclerotinia sclerotiorum* and *Rhizoctonia solani* regardless of the OD or SL storage time.

In comparison with OD, the mild inhibitory effects of SL might be attributed to fewer bioactive components in SL. The biocontrol agents against the pathogenic fungi might have settled with the suspended solids or colloids during centrifugation. Further studies are needed to investigate the fungicidal mechanism.

CONCLUSIONS

Digestate from a biogas plant with pig manure feedstock showed inhibitory effects on the *in vitro* mycelial growth of phytopathogenic fungi, including *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizotonia cerealis*, *Bipolaris sorokinianum*, and *Rhizoctonia solani*. The inhibitory effects of digestate on fungal growth decreased after centrifugation and storage. Further studies should investigate the fungicidal mechanism of the digestate, which could provide a value-added alternative use for such digestate.

Table 3. Colony sizes of crop fungi (mean ±SD) on supernatant liquid-treated media.[a]

Phytopathogen	Digestate Storage Time (d)	Diameter of Fungal Colonies (cm) at Different Post-Digestate Application Times						
		1 d	2 d	3 d	4 d	5 d	6 d	7 d
Fusarium oxysporum	0	1.36 ±0.12**	2.06 ±0.21**	2.66 ±0.23**	3.21 ±0.24**	3.83 ±0.22**	4.16 ±0.23**	4.74 ±0.50**
	7	1.35 ±0.08	2.43 ±0.09*	3.30 ±0.12**	3.98 ±0.12**	4.61 ±0.13**	5.33 ±0.12**	5.90 ±0.22**
	14	1.28 ±0.05**	2.28 ±0.13**	3.03 ±0.11**	3.69 ±0.14**	4.29 ±0.19**	4.87 ±0.17**	5.39 ±0.21**
	28	0.95 ±0.12	1.78 ±0.48	2.58 ±0.65	3.28 ±1.00	3.80 ±1.19	4.41 ±1.47	5.09 ±1.66
Sclerotinia sclerotiorum[b]	0	0.54 ±0.10**	1.09 ±0.65**	2.47 ±1.36**	-	-	-	-
	7	0.50 ±0.00**	2.83 ±0.74**	4.72 ±2.31*	-	-	-	-
	14	0.51 ±0.03**	2.39 ±0.61**	6.14 ±1.16*	-	-	-	-
	28	0.50 ±0.00	0.50 ±0.00**	0.50 ±0.00**	-	-	-	-
Rhizotonia cerealis	0	0.66 ±0.09**	0.88 ±0.28**	1.15 ±0.48**	1.35 ±0.48**	1.45 ±0.55**	1.59 ±0.64**	1.90 ±0.83**
	7	0.50 ±0.00	0.64 ±0.06**	0.83 ±0.24**	1.07 ±0.53*	1.17 ±0.70**	1.33 ±0.94**	1.44 ±1.17*
	14	0.50 ±0.00**	0.64 ±0.13**	0.85 ±0.36**	1.06 ±0.48**	1.24 ±0.55**	1.48 ±0.79**	1.62 ±0.92**
	28	0.50 ±0.00**	0.50 ±0.00**	0.50 ±0.00**	0.50 ±0.00**	0.55 ±0.09**	0.88 ±0.43**	0.94 ±0.51**
Bipolaris sorokinianum	0	1.02 ±0.05**	1.54 ±0.15**	2.22 ±0.36**	2.58 ±0.23**	3.03 ±0.29**	3.41 ±0.24**	3.95 ±0.24**
	7	0.71 ±0.06**	1.37 ±0.17**	1.74 ±0.54**	2.11 ±0.78**	2.24 ±0.95**	2.49 ±1.25**	2.54 ±1.24**
	14	0.54 ±0.03**	1.07 ±0.25**	1.79 ±0.37**	2.38 ±0.46**	3.04 ±0.50**	3.68 ±0.51**	4.42 ±0.67**
	28	0.50 ±0.00**	0.86 ±0.18**	1.42 ±0.69*	1.98 ±1.00*	2.34 ±1.34*	3.08 ±1.52	3.79 ±1.80
Rhizoctonia solani[b]	0	1.22 ±0.16**	1.84 ±0.18**	4.25 ±2.10*	-	-	-	-
	7	0.85 ±0.12**	2.17 ±0.28**	3.85 ±1.47**	-	-	-	-
	14	0.77 ±0.14**	2.93 ±0.51**	6.34 ±0.92**	-	-	-	-
	28	0.53 ±0.06**	1.56 ±0.25**	2.79 ±0.59**	-	-	-	-
Exserohilum turcicum	0	0.52 ±0.01*	0.60 ±0.12	0.69 ±0.25	0.77 ±0.30	0.87 ±0.34	1.04 ±0.46	1.18 ±0.52
	7	0.64 ±0.08	0.71 ±0.14	0.85 ±0.22	0.87 ±0.24	0.84 ±0.26	0.84 ±0.26	0.84 ±0.26
	14	0.58 ±0.10	1.32 ±0.33*	1.85 ±0.39	2.30 ±0.62	2.66 ±0.77	2.81 ±0.71	2.93 ±0.74
	28	0.50 ±0.00**	0.70 ±0.17**	1.07 ±0.55*	1.25 ±0.75*	1.33 ±0.74**	1.67 ±1.06*	1.91 ±1.19
Bipolaris maydis	0	0.59 ±0.04*	0.92 ±0.08	1.16 ±0.19	1.30 ±0.23	1.41 ±0.28	1.55 ±0.32	1.62 ±0.35
	7	0.61 ±0.01	0.80 ±0.05	1.07 ±0.07	1.20 ±0.10	1.30 ±0.13	1.42 ±0.23	1.48 ±0.25
	14	0.53 ±0.02	0.82 ±0.03	1.07 ±0.07	1.30 ±0.08	1.40 ±0.10	1.53 ±0.11	1.67 ±0.12
	28	0.53 ±0.03*	0.75 ±0.10*	0.97 ±0.24*	1.26 ±0.28	1.46 ±0.33	1.65 ±0.35	1.83 ±0.45

[a] Significance: * = significant difference between SL-treated media and the control at $p < 0.05$; ** = highly significant difference between SL-treated media and the control at $p < 0.01$.

[b] Trials were suspended soon after the fungal colonies of the control group spread over the whole Petri plate.

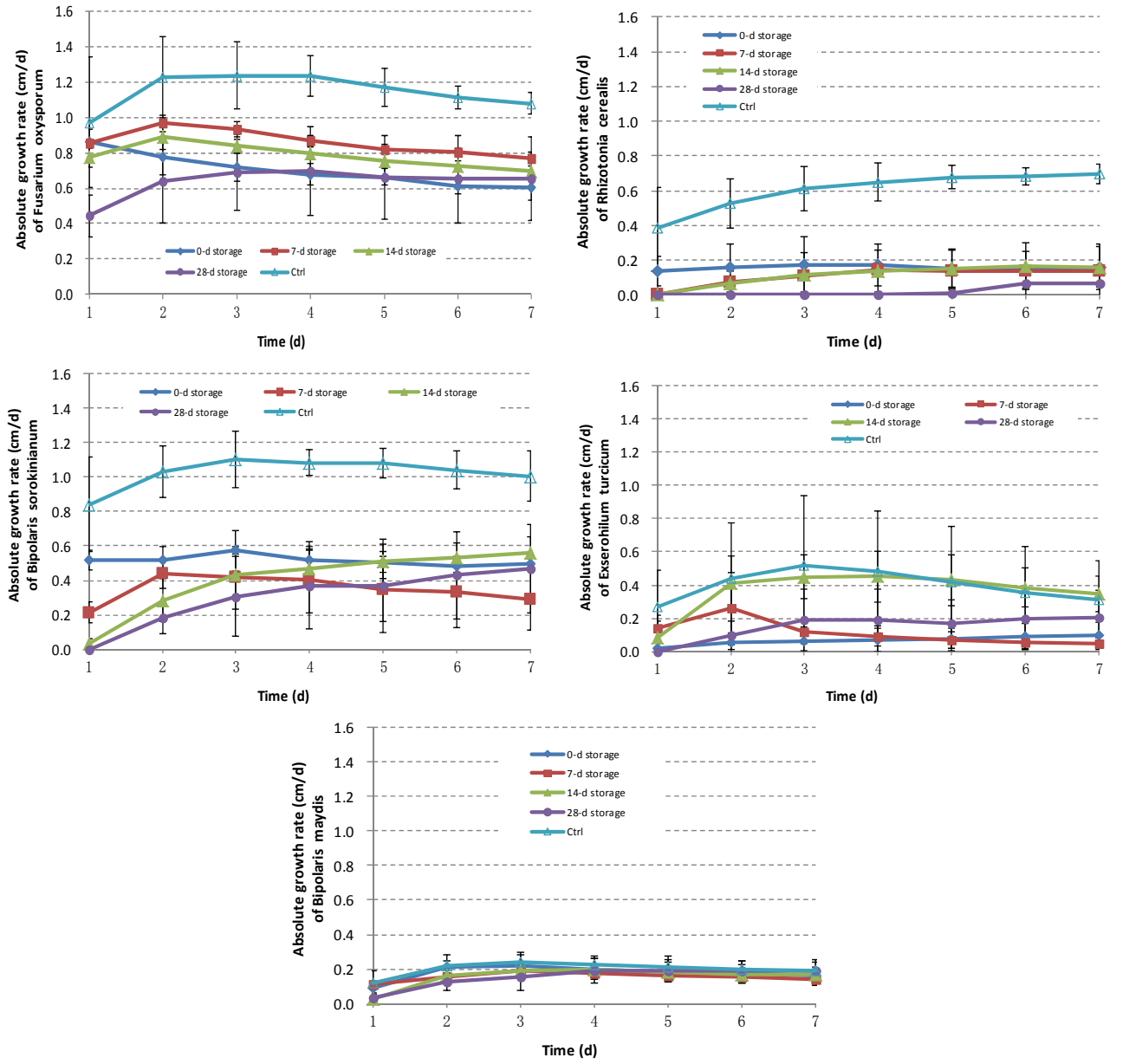

Figure 2. Profiles of absolute growth rates (AGR) of *Fusarium oxysporum*, *Rhizotonia cerealis*, *Bipolaris sorokinianum*, and *Exserohilum turcicum*, and *Bipolaris maydis* on supernatant liquid-treated media during the trial period.

Table 4. Comparison of growth coefficients (k, d^{-1}) of phytopathogens on media supplemented with original digestate (OD) versus supernatant liquid (SL) at different storage times.[a]

	Growth Coefficient (k, d^{-1}) at Different Digestate Storage Times							
	0 d		7 d		14 d		28 d	
Phytopathogen	OD	SL	OD	SL	OD	SL	OD	SL
Fusarium oxysporum	1.195 B	10.746 A	1.227 B	2.304 A	1.569 B	2.276 A	0.804 B	1.061 A
Sclerotinia sclerotiorum	0.376	0.528	0.612	0.652	0.462	0.587	0.172	0.077
Rhizotonia cerealis	0.099 B	0.574 A	0.311	0.309	0.176 b	0.314 a	0.076	0.127
Bipolaris sorokinianum	0.32 B	1.321 A	0.255 B	1.276 A	0.552 A	0.522 B	0.390 b	0.457 a
Rhizoctonia solani	1.080	1.027	0.783	0.808	1.667	0.677	0.852	0.549
Exserohilum turcicum	0.246	0.228	0.353	0.419	0.511 B	0.707 A	0.511	0.377
Bipolaris maydis	0.169 B	0.784 A	0.388 B	0.619 A	0.443 B	0.498 A	0.562 A	0.398 B

[a] Different lowercase letters (a and b) indicate a significant difference ($p < 0.05$) in absolute growth rate (AGR) between OD-treated media and SL-treated media for a given storage time and species. Different uppercase letters (A and B) indicate a highly significant difference ($p < 0.01$) in AGR between OD-treated media and SL-treated media for a given storage time and species.

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